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16. ABSTRACT

Lethal and sublethal effects of chlorination to adult oysters (Crassostrea virginica) were observed during chronic exposures on a seasonal basis. Results show that chronic exposures of oysters to chlorine-produced oxidants (CPO's) can produce mortality at high concentrations and severe sublethal effects at lower levels. Toxicity varies from season to season and is related to seasonal changes in measured CPO concentrations, temperature, and the physiological condition of the oyster. Sublethal effects appear to be related to reductions in feeding and increased avoidance of CPO's. This results in reduced tissue production, causing severe reductions in the size of gonadal tissues and increased dependence in glycogen reserves during exposures to CPO's.

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SEASONAL CHRONIC TOXICITY OF CHLORINATION TO THE AMERICAN OYSTER, *Crassostrea virginica* (G)

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INTRODUCTION

Discharge of chlorine into estuarine and coastal waters may result in undesirable toxic effects to many of the organisms residing in these habitats.[1-3] There is a substantial volume of information on short-term chlorination toxicity in marine invertebrates and fishes;[4-8] however, little is known about the long-term effects of chlorination on the American oyster, *Crassostrea virginica* (G). Galtsoff[9] reports that adult *C. virginica* showed reduced pumping rates at concentrations of chlorine of less than 0.05 mg/l. At concentrations of 1.00 mg/l or greater, oysters closed their valves and ceased to pump; the longest period of exposure, however, was only 48 hr.

Recent work of Bongers *et al.*[10] reports little mortality in adult *C. virginica* exposed to chlorine (0.35-0.85 mg/l) and bromine

chloride (BrCl) (0.17-0.86 mg/l) for 15 days. Sublethal responses revealed that new shell growth was greater in controls than in chlorine and BrCl-exposed oysters, with the amount of shell deposition decreasing with increased toxicant concentrations. No significant difference in the reduction of new shell deposition occurred in tests with chlorinated and BrCl-treated effluents.

A study of the acute toxicity of chlorination to molluscan larvae by Roberts *et al.*[11] revealed that chlorination is very toxic to both oyster (*C. virginica*) and clam (*Mercenaria mercenaria*) larvae. The 48-hr median effective concentration (EC_{50}) was estimated to be less than 0.005 mg/l for larval oysters and 0.006 mg/l for larval clams. The estimated 96-hr EC_{50} (by shell deposition) for juvenile oysters was 0.023 mg/l. Oyster larvae survival under intermittent chlorination was much higher than for continuous chlorination.

Products formed from chlorination of natural waters are a function of physical and chemical parameters of the water, including but not limited to temperature, pH, ammonia, sunlight (UV) and salinity (or the amount of bromine available as a reaction component). Since sea water typically contains 60 mg/kg bromide, bromination rather than chlorination may predominate as salinity increases. Thus, the chlorine-produced oxidant (CPO)[12] levels measured during this study may include varying proportions of hypochlorous acid and hypochlorite ion, as well as hypobromous acid and hypobromite ion, depending upon the ambient salinity. In addition, photolysis may influence the level of bromate produced during chlorination of saline waters.[13]

The objective of this study was to examine the effects (lethal and sublethal) of chlorination to adult oysters, *Crassostrea virginica,* during chronic exposures on a seasonal basis.

MATERIALS AND METHODS

Bioassays were conducted during the fall (45-day exposure), winter (75-day exposure) and spring (60-day exposure) of 1976-1977. Adult oysters (5.0-12.0 cm height) were collected from Leadenwah Creek (coordinates N 32° 36′ latitude, W 80° 15′ longitude), a large tidal tributary of the North Edisto River

Estuary, and transported immediately to the lab where they were scrubbed and all fouling organisms removed. After numbering and measuring individual heights, 50 oysters were placed in each of four exposure tanks (110 cm x 63 cm x 28 cm) and acclimated in running sea water (> 5.0 liter/hr/oyster) for 15 days. An additional tank was maintained as a control. Each tank was cleaned at least every other day (daily when possible) during both acclimation and the seasonal exposure periods.

During each seasonal bioassay the following measurements were used to assess the effects of chronic exposure to chlorination:

1. Percentage survival was recorded daily.
2. Shell height was measured prior to and at the end of each seasonal exposure using Vernier calipers.
3. Growth was monitored by measuring changes in shell height and weight in 10 oysters/exposure concentration plus controls at day zero and 45 days (fall), 75 days (winter) and 60 days (spring), using the techniques described by Havinga[14] and Andrews.[15]
4. Condition index measurements were made using the method described by Galtsoff[9]:

$$\text{Condition Index} = \frac{\text{Dry Body Weight (g)}}{\text{Cavity Volume (ml)}} \times 100$$

modified as follows. Calculations of cavity volume are usually obtained by subtracting from the total volume of the oyster (including its shell) its empty shell volume as determined by the water displacement method. However, this method may be subject to significant manipulative error and is very time consuming.

In recent work Andrews[15] reported that the specific gravity of oyster tissues is approximately the same as the specific gravity of sea water, 1.022 g/cm^3. Therefore, cavity volume may be determined by subtracting the empty shell weight from the total weight of the oyster (including its shell), since there is approximately a 1:1 ratio of weight-to-volume in the tissues removed. A comparison of cavity volumes derived by both the weight-to-weight method and the volume-to-volume method showed no statistically significant ($P > 0.05$) differences. The weight-to-weight method provides a more rapid and precise manner in

which to determine cavity volume measurements and hence condition indices measurements (Scott, unpublished data).

5. Gonadal index measurements were made using the technique of Galtsoff[9]:

$$\text{Gonadal Index} = \frac{\text{Dry Weight of Gonads (g)}}{\text{Total Dry Body Weight (g)}} \times 100$$

6. Chlorine-produced oxidant (CPO) concentrations (free + combined chlorine and other residual oxidants) were measured with a Wallace and Tiernan Amperometric Titrator using procedures outlined in *Standard Methods*.[16] Phenylarsine oxide and pH buffers used were standard Wallace and Tiernan reagents. The 5% KI solution and chlorine (Fischer NaOCl Analytical Reagent, minimum 5.0% NaOCl) stock solutions were prepared in deionized water. Stock solutions were kept in light-proof containers to prevent photodecomposition. All water samples were taken directly from treatment aquaria at least every 2-3 days during each seasonal experiment. Agitation of samples was avoided to reduce the potential for error by photodecomposition or flashing-off of CPO.[17]
7. The water temperature, salinity, pH and dissolved oxygen concentration in each seasonal experiment were those ambient in the incoming sea water from the North Edisto River Estuary. Mean ambient sea water temperatures were 17.5°C (fall), 11.2°C (winter) and 27.0°C (spring). Mean salinities during the study were 25.4°/oo (fall), 23.3°/oo (winter) and 26.6°/oo (spring). Periodic dissolved oxygen measurements showed that levels were always near saturation. The pH ranged from 7.40-8.30 during the study.
8. Fecal production measurements were made every 2-3 days by observing the number of oysters producing feces per exposure concentration plus controls.

RESULTS

Physical and Chemical Measurements

Measurements of physical [temperature (°C) and salinity (°/oo)] and chemical [nominal NaOCl and measured CPO (mg/l]

parameters are recorded in Table I for each seasonal bioassay. Measured CPO concentrations varied considerably from season to season at each nominal CPO concentration. Measured CPO concentrations are highest during the winter exposure period, decreasing during both the fall and spring. Overlaps in the range between various measured CPO concentrations possibly occurred because of the continually changing organic load in the ambient sea water as well as seasonal changes in water temperature and sudden changes in salinity generally associated with passing weather fronts (usually accompanied by increased water turbulence and rainfall).

Table I. Physical and Chemical Parameters Measured During Each Seasonal Bioassay

Nominal NaOCl Concentration (mg/l)	Measured CPO Concentration (mg/l)		Temperature (°C)		Mean Salinity (‰)
	Range	Mean	Range	Mean	
		Fall			
5.60	0.98 - 3.26	1.58	10.5 - 27.0	17.5	25.4
3.20	0.47 - 1.94	0.91	10.5 - 27.0	17.5	25.4
1.80	0.18 - 0.49	0.28	10.5 - 27.0	17.5	25.4
1.00	0.08 - 0.22	0.14	10.5 - 27.0	17.5	25.4
Control	0.00	0.00	10.5 - 27.0	17.5	25.4
		Winter			
5.60	2.02 - 4.95	2.98	4.0 - 20.0	11.2	23.3
3.20	0.84 - 2.09	1.23	4.0 - 20.0	11.2	23.3
1.80	0.22 - 0.68	0.39	4.0 - 20.0	11.2	23.3
1.00	0.09 - 0.24	0.16	4.0 - 20.0	11.2	23.3
Control	0.00	0.00	4.0 - 20.0	11.2	23.3
		Spring			
5.60	0.75 - 3.82	1.81	20.5 - 31.5	27.0	26.6
3.20	0.39 - 1.20	0.65	20.5 - 31.5	27.0	26.6
1.80	0.11 - 0.34	0.24	20.5 - 31.5	27.0	26.6
1.00	0.08 - 0.17	0.12	20.5 - 31.5	27.0	26.6
Control	0.00	0.00	20.5 - 31.5	27.0	26.6

Seasonal Mortality

The percentage survival of adult oysters to different measured CPO concentrations during each seasonal bioassay is shown graphically in Figures 1 (fall), 2 (winter) and 3 (spring). Survival varied from season to season at each nominal NaOCl concentration.

In the nominal 5.60 mg/l NaOCl concentration, there was a delay in mortality, with 0% survival occurring on day 70 (winter) vs day 22 (fall) and day 32 (spring). This delay in mortality during the winter bioassay occurred when mean measured CPO concentrations were at their highest [2.98 mg/l (winter) vs 1.58 mg/l (fall) and 1.81 mg/l (spring)].

Seasonal survival varied in the nominal 3.20 mg/l NaOCl concentration as well. Lowest survival occurred during the fall bioassay (12% after 45 days). Survival was higher in the winter (44% after 75 days) and much higher in the spring (78% after 60 days). Mortality was delayed in the winter bioassay and

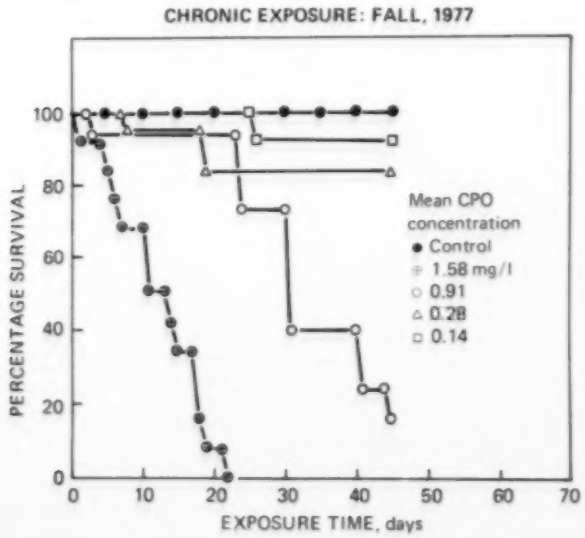

Figure 1. Percentage survival of adult oysters exposed to different concentrations of CPO during the fall seasonal exposure.

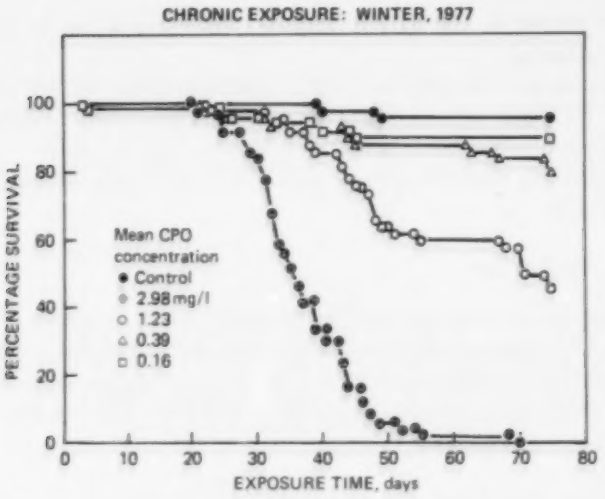

Figure 2. Percentage survival of adult oysters exposed to different concentrations of CPO during the winter seasonal exposure.

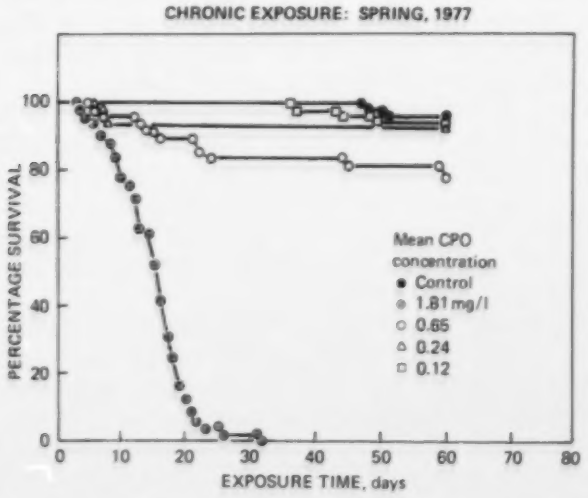

Figure 3. Percentage survival of adult oysters exposed to different concentrations of CPO during the spring seasonal exposure.

nearly absent in the spring bioassay when compared to the fall exposure.

Survival in the nominal NaOCl concentrations of 1.80 mg/l and 1.00 mg/l was much higher in the spring than in the fall and winter bioassays (Figures 1 and 2).

Survival in controls was always above 94% in all seasonal exposures.

Fecal Production

Measurements of seasonal mean fecal production are listed in Table II. Concentrations of 0.12-0.16 mg/l of CPO result in a 50% or more reduction in fecal production regardless of season. Mean fecal production in controls was at least 83% during each seasonal exposure.

Condition Index and Gonadal Index Measurements

Sublethal effects of CPO concentrations on adult oysters as determined by condition index and gonadal index measurements are listed in Table III and depicted graphically in Figures 4, 5, 6 and 7 for winter and spring experiments. The condition index was measured only once for fall oysters which had too little gonadal tissue to estimate a gonadal index.

Condition index measurements in controls were always significantly higher ($P = 0.01$-0.05) than in measured CPO concentrations during each seasonal exposure. There was a general decrease in condition index with increased exposure time at all measured CPO concentrations. Seasonal fluctuations in condition indices in controls are probably related to the storage and release of gonadal material.

Gonadal index measurements closely paralleled condition indices measurements. Control gonadal indices were significantly greater ($P = 0.01$-0.05) than those determined for each measured CPO concentration during each seasonal exposure. There was a general decrease in mean gonadal index with increasing exposure time in all seasonal exposure concentrations of CPO. Peak control gonadal index measurements occurred during the spring exposure (May) followed by a general decrease during late spring and early summer.

Table II. Summary of Mean Seasonal Fecal Production in Adult Oysters Exposed to CPO[a]

Nominal NaOCl Concentration (mg/l)	Mean Measured CPO Concentration (mg/l)	Mean Oyster Fecal Production (%)
	Fall	
5.60	1.58	0
3.20	0.91	0
1.80	0.28	6
1.00	0.14	19
Control	0.00	85
	Winter	
5.60	2.98	0
3.20	1.23	1
1.80	0.39	10
1.00	0.16	39
Control	0.00	83
	Spring	
5.60	1.81	0
3.20	0.65	10
1.80	0.24	26
1.00	0.12	49
Control	0.00	93

[a]Measured CPO values (mg/l) are seasonal means. Fecal production in control oysters was significantly ($P = 0.01$) larger than in exposed oysters during each seasonal exposure.

Growth Measurements

Growth measurements are not reported in this study due to the discovery of a growth layer inside the shell of CPO-exposed oysters which makes interpretation of results difficult. Electron microscopy studies to characterize this growth layer are presently under way at the University of South Carolina. As oysters are exposed to CPO, the mantle apparently becomes irritated and retracts into the foliated layer away from the prismatic edge of the shell. At its new

Table III. Seasonal Mean Condition Index and Gonadal Index Measured in Adult Oysters Exposed to CPO[a]

	Nominal NaOCl Concentration (mg/l)				
	5.60	3.20	1.80	1.00	Control
		Fall			
Measured CPO Concentration (mg/l)	1.58	0.91	0.28	0.14	0.00
45-Day Condition Index					
$\bar{X}$	0.00	1.74	2.52	2.23	3.21
S.d.	0.00	0.799	0.500	0.576	0.738
		Winter			
Measured CPO Concentration (mg/l)	2.98	1.23	0.39	0.16	0.00
30-Day Condition Index					
$\bar{X}$	0.00	5.60	5.69	5.71	7.38
S.d	0.00	1.346	1.669	2.761	2.468
75-Day Condition Index					
$\bar{X}$	0.00	2.66	3.99	4.01	4.92
S.d.	0.00	1.015	1.194	1.036	1.201
75-Day Gonadal Index					
$\bar{X}$	0.00	2.38	3.61	5.10	10.38
S.d	0.00	1.252	2.028	2.821	5.580
		Spring			
Measured CPO Concentration (mg/l)	1.81	0.65	0.24	0.12	0.00
30-Day Condition Index					
$\bar{X}$	0.00	2.75	3.91	3.94	5.08
S.d.	0.00	0.603	1.203	0.962	1.465
30-Day Gonadal Index					
$\bar{X}$	0.00	9.14	19.32	20.44	30.83
S.d.	0.00	9.976	10.639	8.554	9.644
60-Day Condition Index					
$\bar{X}$	0.00	3.29	3.59	3.00	4.60
S.d.	0.00	1.049	0.755	0.966	1.112
60-Day Gonadal Index					
$\bar{X}$	0.00	12.87	12.04	9.33	23.65
S.d.	0.00	4.317	6.161	5.443	10.180

[a]All measurements of condition index and gonadal index in oysters exposed to CPO are significantly smaller ($P = 0.01$-0.05) than those measurements in control (unexposed) oysters, during each seasonal bioassay. $N = 20$ (number of oysters/measurement) in each mean value reported. Reported CPO values (mg/l) are seasonal means.

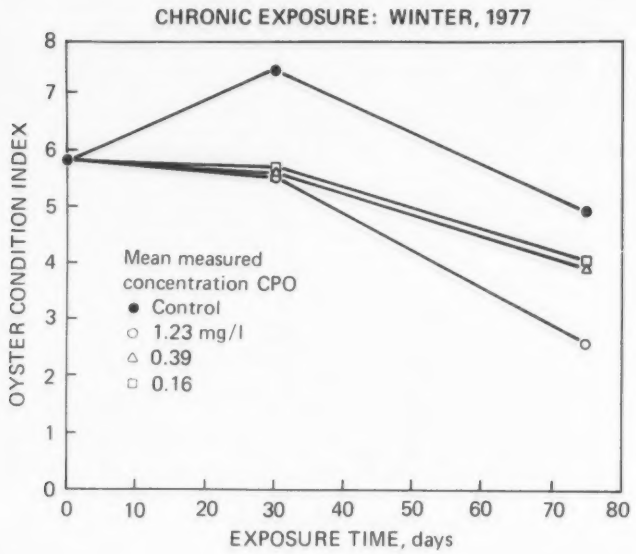

Figure 4. Mean condition index measurements in adult oysters exposed to different CPO concentrations (mg/l) during the winter bioassay (75 days exposure).

position farther inside the shell, the mantle then lays down new shell growth upon the older foliated layer of the shell. This new shell growth appears to be different from the normal growth noted in either the foliated or prismatic portions of the shells in controls.

DISCUSSION

Our data suggests obvious seasonal differences in the percentage survival at the various CPO concentrations, especially the nominal 5.60 and 3.20 mg/l levels. The delay in mortality in the nominal 5.60 mg/l NaOCl level during the winter bioassay when measured CPO concentrations were the highest (2.98 mg/l) indicates that other factors, such as temperature and the physiological condition of the oyster may be important in determining the observed mortality patterns. The initial 30-day delay in mortality in the winter bioassay in the measured CPO

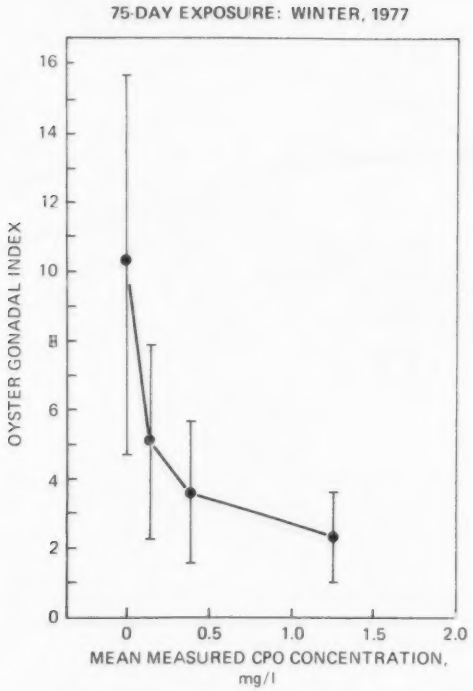

Figure 5. Mean gonadal index measurements in adult oysters exposed to CPO (mg/l) for 75 days. Bars (I) indicate one standard deviation unit around mean.

concentration of 2.98 mg/l (see Figure 2) corresponds with a time period in which water temperatures were 5°C or below. According to Galtsoff,[18] oxygen uptake decreases markedly at temperatures below 5°C, with the valves often being closed at this low temperature and ventilation of the gills being stopped. This closure of the valves and lowered metabolic rate may prevent the oyster from being exposed to the CPO until water temperatures increase, causing elevated metabolic rates, increased gill ventilation and more direct exposure to the toxicant.

A previous study by Middaugh *et al.*[19] has shown that exposure of juvenile spot, *Leiostomus xanthurus*, to CPO with increasing temperature caused increased toxicity. Thatcher *et al.*[20]

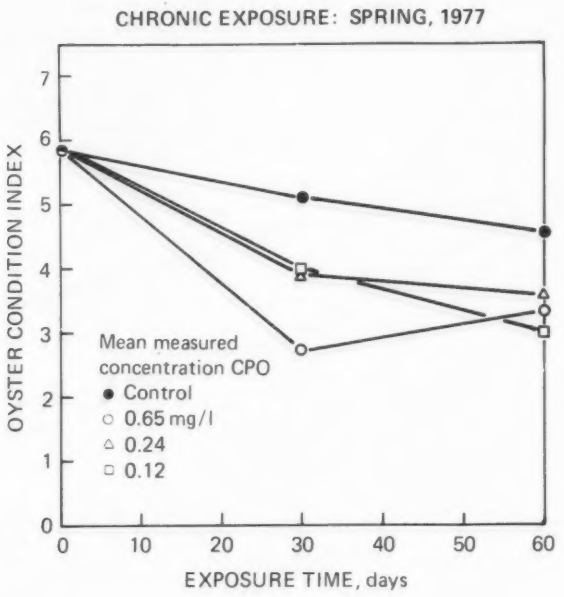

Figure 6. Mean condition index measurements in adult oysters exposed to different CPO concentrations (mg/l) during the spring bioassay (60 days exposure).

have also shown the possible synergistic effects of increasing temperature and chlorine on the survival of juvenile brook trout, *Salvelinus fontinalis.*

A similar temperature effect is obvious for oysters if the time to 100% mortality is compared in the winter bioassay (70 days) to that found in fall and spring (22 days and 32 days, respectively) in the nominal 5.60 mg/l NaOCl concentrations. A comparison of the mean measured CPO concentrations reveals highest concentrations were measured during the winter, 2.98 mg/l [vs 1.58 mg/l (fall) and 1.81 mg/l (spring)], when time to 100% mortality was delayed. These data strongly support our conclusion that seasonal factors such as water temperature and the physiological condition of the oysters are contributing factors in their response to CPO.

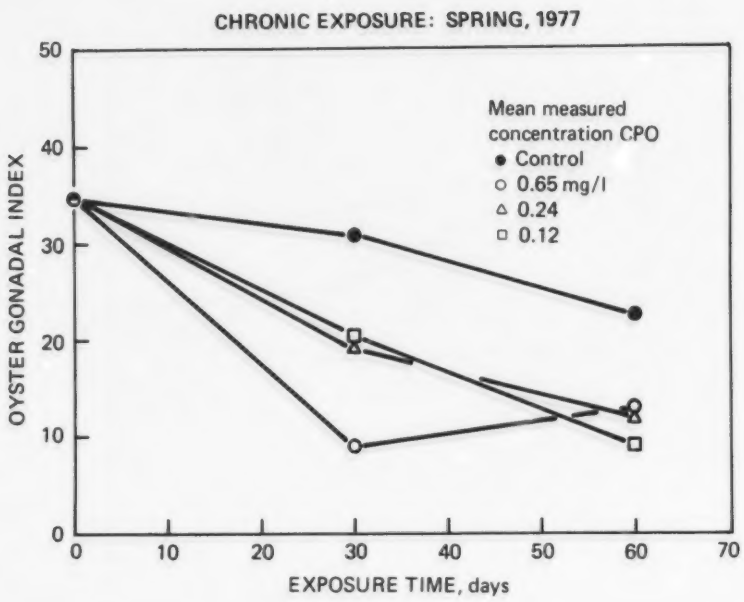

Figure 7. Mean gonadal index measurements in adult oysters exposed to different CPO concentrations (mg/l) during the spring bioassay (60 days exposure).

Differences in the percentage survival observed in the nominal 3.20 mg/l concentration are also variable and again may reflect the seasonal fluctuations in mean measured CPO concentrations [0.65 mg/l (spring), 0.91 mg/l (fall) and 1.23 mg/l (winter)], water temperature and physiological condition of the oysters tested. Comparisons of time to death revealed striking seasonal differences. In the fall bioassay there was 88% mortality in 45 days compared to 24% during the winter and 20% during the spring. The increased mortality in the fall is probably related to the physiological state of the oysters during this season of the year. Mean condition index of control oysters during the fall was 3.21 vs 7.38 during the winter and 5.08 during the spring. The lower fall condition index measurement is probably related to postspawning effects. Our data suggest that this physiological state may make the oyster more susceptible to the toxic effects of chlorination.

The percentage survival of oysters at nominal NaOCl concentrations of 1.80 and 1.00 mg/l is much greater than those observed at the higher exposure concentrations. Although seasonal differences in percentage survival did occur these differences were not significant, as at the higher chlorination levels. A recent study by Bongers *et al.*[10] reported 92-100% survival in oysters, *C. virginica,* exposed to chlorination concentrations of 0.35-0.85 mg/l for 15 days. Our data show a similar percentage survival of 92-100% after only a 15-day exposure, but as exposure time increased the percent survival decreased substantially during each seasonal bioassay. For example, during the winter experiment, percentage survival after a 15-day exposure to a mean measured CPO concentration of 0.39 mg/l was 94% compared to 80% survival after 75 days. Control survival during this same time period was 96% (for 75 days).

Results of seasonal measurements of fecal production indicate significant reductions in fecal production with increasing CPO concentrations. However, there were no observed seasonal differences in oysters exposed to different CPO concentrations. Our data suggest that the presence of CPO products in the sea water alters the production of fecal material. This infers some effect upon feeding. Recent studies by Gentile *et al.*[21] have shown that a CPO concentration of less than 1.00 mg/l was responsible for complete mortality of all phytoplankton species in Narragansett Bay, Rhode Island, waters. The reduction in the ability of the oyster to produce feces (hence feed) may be related to its refusal of chlorine-exposed algae or an avoidance reaction induced by the irritability of CPO products in sea water (*i.e.*, Galtsoff[9] reports oysters closed valves when chlorine concentrations were $\geqslant$ 1.00 mg/l), or a combination of both.

Seasonal condition index measurements indicated significant ($P = 0.01$-0.05) reductions in tissue production in oysters exposed to CPO concentrations. Reductions in tissue production appear to be most severe with increasing lengths of exposure and increasing concentrations of CPO. Our data suggest that the exposed oysters close their valves and rely upon body food reserves. This would mean a possible shift to anaerobic metabolic pathways.[18,22]

Seasonal gonadal index measurements firmly support seasonal condition index measurements. There are significant (P = 0.01-0.05) reductions in seasonal gonadal indices with increasing lengths of exposure and increasing CPO concentrations when compared to control values. The most severe effect was found during the spring when gonadal development is at its peak. Gonadal index data from our spring bioassay (Figure 7) confirm our observation of reduced tissue production in our spring condition index measurements (Figure 6) and support the idea that tissue reduction must occur in the glycogen reserves of the gonads. This strongly supports our earlier contention of increased dependence upon body food reserves and implies the gonads may be the tissues most critically affected.

CONCLUSIONS

Results of this study have shown that chronic exposures of *Crassostrea virginica* to CPO products can produce considerable mortality at high concentrations and severe sublethal effects at lower levels. Toxicity varies from season to season and is related to seasonal changes in measured CPO concentrations, temperature and the physiological condition of the oyster. Sublethal effects appear to be related to reductions in feeding and increased avoidance of CPO. This results in reduced tissue production causing severe reductions in the size of gonadal tissues as well as increased dependence upon glycogen reserves during exposures to CPO.

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